1: Pigment Cell Res. 1993 Dec;6(6):394-9. Related Articles, L

Improved tyrosinase activity stains in polyacrylamide electrophoresis gels.

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Mammalian tyrosinase exists in a variety of subcellular locations and maturation states that result from a complex post-translational processing wit possible regulatory implications. So far, SDS-PAGE has proven to be the method of choice for the resolution of tyrosinase isoforms. However, the relatively poor sensitivity of the currently available specific activity stain bas on incubation of the gels with L-dopa until the formation of melanin has severely limited the use of electrophoresis in regulation studies. Two alternat staining procedures are presented and discussed. The first one involves the fluorographic detection of radioactive melanin after incubation of the gels in the presence of L-[3-14C]-dopa. A similar method has already been used by others (Tsukamoto et al., 1992, Pigment Cell Res. [Suppl.] 2:84-89), but its performance has not yet been compared to the one of the dopa procedure. Th sensitivity of this method can be varied by adjusting the isotopic dilution of t tracer and/or the time of exposure of the gel, but it is at least ten times higher than the one of the colorimetric stain. Moreover, the intensity of the bands is proportional to the initial tyrosinase activity over a wide range. Using this procedure, the activity present in the different subcellular fractions of melanocytes in culture can be easily detected. The second procedure involve the formation of a colored adduct between dopaquinone and MBTH. (ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 7511805 [PubMed - indexed for MEDLINE]

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1: Int J Hyperthermia. 2002 Nov-Dec;18(6):563-75.

MetaPress

Heat shock protein 70: role in antigen presentation and immune stimulation.

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Heat shock proteins (HSP) when released into the extracellular milieu can ac simultaneously as a source of antigen due to their ability to chaperone peptid and as a maturation signal for dendritic cells, thereby inducing DCs to cross-present antigens to CD8+ T-cells. HSP can also act independently from associated peptides, stimulating the innate immune system. Previous results regarding the activation of NK cells by HSP70 cell surface expression on tumour cells and soluble HSP70 will be further covered elsewhere within thi issue. For cross-presentation, HSP70-peptide complexes (HSP70-PC) were used from two human melanoma cell lines that differ in the expression of the tumour-associated antigen tyrosinase. Purified HSP70-PC consists of both th constitutively expressed HSC70 and the inducible HSP70. HSP70-peptide complexes purified from tyrosinase positive (HSP70-PC/tyr+) human melanoma cells, incubated with immature DCs, results in the activation of HLA-*A0201-restricted tyrosinase peptide-specific T-cells. Receptor-mediat uptake of HSP70-PC by DCs and intracellular transport are required for efficient MHC class I restricted cross-presentation of chaperoned peptides. Demonstration of HSP70-PC mediated cross-presentation of such non-mutat naturally expressed tumour antigens is of special clinical interest with regard hyperthermia. Tumour regression and improved local control have been shov within clinical phase II/III trials integrating regional hyperthermia combined with radiation and/or chemotherapy in multimodal treatment strategies. According to the proposed concept, local necrosis induced by hyperthermic treatment induces the release of HSPs, followed by uptake, processing and presentation of associated peptides by DCs. By acting as chaperone and a sig for DC maturation, HSP70-PC might efficiently prime circulating T-cells. Therefore, upregulating HSP70 and causing local necrosis in tumour tissue b hyperthermia offers great potential as a new approach to directly activate the immune system.